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Amino Acid Supplementation

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Amino Acid Supplementation

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Introduction

As more and more nutritional surveys are being conducted in the technically underdeveloped areas of the world, it is becoming increasingly clear that the most significant nutrient lack is protein — especially protein of high biological value. The great challenge for the nutritionist, the agronomist, and the political scientist is to provide the ways and means of improving the diets of the underprivileged with respect to a more adequate protein intake. Such an accomplishment would help to eradicate much disease and provide greater vigor and health to literally millions of people.

Through basic research efforts resulting in relatively inexpensive sources of amino acids, a valuable new tool — *amino acid supplementation* — is made available for improving the nutritional quality of proteins.

The basic problems of amino acid supplementation are numerous with many as yet unsolved, but solutions are gradually being achieved through research which will be reviewed here.

The nutritional significance of the amino acids has been discussed in an earlier issue of the Borden's Review (1). Suffice it to say here that the pioneer work of W. C. Rose (2) culminating in the discovery of threonine has enabled him and others to classify amino acids according to their indispensibility for certain life processes. Furthermore, under carefully defined conditions, the quantitative requirements for each of these essential amino acids has been established. These requirements have been found to vary rather widely as does the amino acid content of proteins from various sources. For these reasons it is obvious that supplementation of some proteins with certain of the indispensable amino acids should improve the quality of the protein out of proportion to the quantity of material added. In general, the proteins from animal sources with the exception of the collagens contain high percentages of the essential amino acids in proper ratios and therefore are excellent food sources for man and animals. The vegetable proteins, on the other hand, have as a rule a lower content of essential amino acids and moreover are more poorly balanced with respect to the several constituent amino acids. Further it has been found that some proteins are partially resistant to enzymatic attack (3, 4). Since the several amino acids must be absorbed at essentially the same time for maximum availability for tissue synthesis (5, 6, 7, 8) this fact undoubtedly contributes to some extent to the poor biological value of some of the vegetable proteins. Proteins of vegetable origin are used in feeding more than two-thirds of the world's human population

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and almost all of its domesticated animals and it is evident that there is a vast potential for amino acid supplementation of vegetable proteins.

Availability of Amino Acids

Until recently crystalline amino acids have been available from two sources only. They were either isolated from acid, alkali or enzymatic hydrolysates of proteins or were synthesized. In the latter case, since all of the proteinogenic amino acids, with the exception of glycine, contain at least one center of assymetry, mixtures of optical isomers are obtained and these must be used as such or separated by what in the past have often been laborious resolution procedures.

Now a third source of some of the amino acids is available and may eventually prove to be more important than either of the others. This is production by microorganisms. This method has the advantage that the free L-amino acid is produced from simple source materials relatively free from all other amino acids with the result that isolation is easy and no resolution is necessary. At present excellent procedures are available for the production of L-lysine (9) and L-glutamic acid (10, 11, 12, 13).

It is obvious that production of amino acids from protein hydrolysates is not a very likely source for large scale supplementation. If a protein which has a high content of some desirable amino acid can be produced why not feed this protein alone or in combination with others which are mutually supplementary? Indeed this practice is used widely and is one of the best and cheapest means of upgrading a substandard diet. On the other hand, there may be a few cases in which a by-product protein is not satisfactory for feed supplementation but may serve as a source of an amino acid or a group of amino acids. At the present time blood meal falls in this category. It has not proved entirely satisfactory as a feed additive and has been used largely as a fertilizer. Its high content of basic amino acids makes it possible to isolate them at low cost individually or as a mixture. Even such a source as this, however, is not likely to be of any great importance in considering supplementation of the diet of large segments of the world's population.

A large amount of research has gone into discovery of new routes of synthesis of the amino acids with the result that many of them can now be produced at a fraction of their cost of a few years ago. Methods of resolution have also been greatly improved. For animal feeds resolution would not appear to be necessary since the D-forms of the amino acids when not utilized as essential amino acids do contribute some non-specific nitrogen. The toxicity of the D-forms is in some cases at least, less than that of the L-isomers (14). For purposes of animal feed supplementation, use of racenic amino acids would probably depend upon whether or not economical procedures could be found for converting the D to the L-forms. In human food where taste, bulk and texture are more important factors, only L-amino acids are likely to find widespread use.

Given sufficient incentive in the way of a large market it is estimated that any of the L-amino acids might eventually be synthesized to sell for from 1 to 3

dollars per pound. With an adequate mutation program, production by fermentation at less than one dollar per pound is within the realm of possibility.

Amino Acid Imbalance

It has aptly been said that we are now emerging from a period in which the chief aim of nutritionists has been the identification of the essential metabolites. How well we have succeeded in this phase of the science is clearly shown by a recent report of M. O. Schultze (15)) in which he describes the maintenance of healthy rats through three generations on a diet of known chemical composition. This does not mean that no new factors will be discovered. The lactational performance of Schultze's animals was below par indicating that some unknown factor may be involved in this important function. In addition, since several of the known essential factors are produced by bacteria in the intestine and absorbed therefrom it is not unlikely that as yet unidentified substances may be supplied in the same manner. Further, other unknown factors may be involved in resistance to infection and to other stressing agents. The fact remains, however, that animals can grow and reproduce on a chemically defined diet.

Having established most of the requirements of the animal organisms, scientists set about to investigate their metabolic pathways and interrelationships. While this phase of the work is still in its infancy, one fact has emerged with startling clarity: All factors are complexly interrelated! In studying any one essential factor one must take into account every other compound required by the animal under study.

Nowhere is this more true than in the field of amino acid metabolism. As early as 1939 Gladstone (16) reported antagonisms in bacteria between certain of the amino acids. Considering the close structural relationship between these compounds such antimetabolic action is not surprising. What has proved surprising are the very delicate balances that exist in higher animals between one amino acid and another and between amino acids and other essential nutrients. Such interrelationships must be taken into consideration when one is contemplating altering the amino acid composition of a diet by supplementation.

Excellent reviews (17, 18, 19) have been prepared on this subject and therefore only a few of the many examples will be discussed. A most interesting study involving both amino acids and a vitamin arose from the observation by Krehl et al that the addition of corn to a casein-containing diet caused an inhibition in the growth of rats and that either tryptophan or niacin would reverse this effect (20). It was soon found by these workers that other tryptophan deficient proteins or protein hydrolysates inhibited growth which inhibition was again reversed by tryptophan or niacin (21). It developed then that the inhibitory effect of proteins or protein hydrolysates could be duplicated by adding 0.078% threonine or 0.28% phenylalanine to a diet containing 9% casein and 0.2% cystine (22). Elimination of the cystine eliminated the effect of the threonine (23). Methionine gave the same results as cystine in this system.

Following this lead, Henderson et al (24, 25) went on to demonstrate an extremely delicate balance between tryptophan and several of the essential amino acids. Rats receiving purified amino acid diets containing 0.10 to 0.11% tryptophan develop a niacin deficiency when all the remaining essential amino acids are present at levels approaching those required for optimum growth. If the leucine, isoleucine, valine, threonine or lysine content of the diet are reduced so that any of these amino acids become more limiting than tryptophan, growth is improved indicating that more tryptophan is available for niacin synthesis with a correction of the niacin deficiency.

The improvement of the nutritional value of corn by heating with water or lime offers an excellent example of the extreme complexity of foodstuff interrelationships. Kodicek (26) and Pearson *et al* (27) present convincing evidence that no free niacin exists in untreated corn. Since heating does effect release of a portion of this vitamin it is tempting to assume that the beneficial effect of heat is to increase the supply of unbound niacin. On the other hand, heat treatment is effective even when ample niacin is included in the diet. However, Henderson and co-workers (28) have shown that rats on a niacin deficient diet grow much more rapidly with heat treated corn starch as a source of carbohydrate than with untreated corn starch itself. Nonetheless Laguna and Carpenter (29) found that the beneficial effect of heat treatment could be realized by heating the non-starchy portions of the grains and not by treatment of the corn starch alone. This finding lends credence to the contention of Bressani (30) that heat or lime treatment are effective because they destroy portions of certain of the essential amino acids of the alcohol soluble zein and in this manner bring about a more satisfactory amino acid balance. One is tempted to suggest that there are still unexplored interrelationships affecting the nutritive value of corn. According to Rose's requirements for the growing rat, lysine is the most limiting amino acid. Nonetheless supplementation with lysine, or lysine plus a source of non-specific nitrogen does not improve growth on a niacin containing diet. Whether this can be explained by failure of digestion remains to be demonstrated.

Fox, Briggs and Ortiz (31) have recently reported another interesting vitamin-amino acid relationship. In chicks receiving a purified diet, methionine was found to eliminate completely the requirement for vitamin B_{12}. Free methionine alone was more effective in sparing vitamin B_{12} than an equivalent amount of methionine in casein or in an amino acid mixture patterned after casein. This same amino acid mixture without methionine markedly depressed growth below that of the group receiving no vitamin B_{12}. Addition of the vitamin produced optimal growth.

Harper and his associates (32, 33, 34) have made a careful study of the effect of amino acids on deposition of liver fat in rats maintained on a low protein diet. On a diet containing 9% casein, 5% corn oil and 81.6% sucrose weanling rats deposit large quantities of fat in their livers. These organs may contain 25-35% fat on a dry weight basis compared to 12-16% encountered with a good high protein diet. No pathology is associated with this effect and

the liver fat returns to normal as the animal approaches maturity. It is, however, a significant phenomenon and offers another means of studying amino acid relationships. Six per cent gelatin, 2% casein or 0.18% DL-threonine are effective in reducing the accumulation of liver fat. Serine, glycine, glycocyamine, choline and betaine are less effective. The authors suggest that three types of lipotropic substances exist: 1) Choline for phospholipid synthesis, 2) Threonine and probably other amino acids for enzyme formation, and 3) Non-specific factors such as glycine, betaine and serine.

Despite the fact that by calculation from amino acid analysis, lysine is the most limiting amino acid in rice with threonine not the second, but the fifth most limiting (35). Pecora and Hundley (36) have showed that both lysine and threonine were required to supplement a rice diet to enable it to support reasonably good growth in rats. The Wisconsin workers have also studied the effect of amino acid supplementation of diets containing a high content of cereals such as might be used by large segments of the population of technically underdeveloped areas. They found for instance, that an unsupplemented rice diet gave poor growth and high liver fat (37). The inclusion of 0.2% L-lysine hydrochloride and 0.24% of DL-threonine produced a very significant increase in growth rate but had no effect on liver fat. Increasing the lysine supplement to 0.4% reduced the liver fat to normal but also caused a reduction in growth which now could be overcome only by increasing the levels of leucine, isoleucine, valine and histidine (35).

Deshpande, Harper and Elvehjem (34) reported that the rate of growth of young rats increased from 3 to 21 grams per week when the diet containing 78% white flour was supplemented with 0.5% L-lysine and 0.4% DL-threonine. Although lysine was limiting for growth, liver fat did not accumulate on the diet containing 78% flour. However, when the flour was fed at a protein level equivalent to that of rice (44.2% flour), fatty infiltration occurred and was prevented by lysine supplementation. Shils and Stewart (38) have shown that the fatty livers resembling those found in Kwashiorkor which are produced in rats fed corn, cassava or rice diets may be partially or completely prevented by supplementation with lysine and tryptophan. These observations and others not enumerated here lead one to conclude that *it is only on relatively low protein diets that delicate amino acid imbalances occur*. They also lead us to the inescapable conclusion that amino acid imbalances do exist in nature. Nothing could be more logical than to correct them when possible.

Protein Reserves

The occurrence of acute amino acid imbalance on low protein diets only, leads us to a consideration of protein reserves. It has been generally taught that there are no protein depots in the same sense as the fat depots which serve as reserves and nothing more. This may be true but it is also true that some tissue proteins are much more available than others to meet the stress of inanition. In rats on a protein-free diet a high percentage of liver protein disappears in a very few days.

Although all proteins may have other functions, there appears to be a set order of priority to be followed in the raiding of tissues for amino acids for repair of such indispensable tissue as that of the heart.

It was established some years ago that the reabsorption of some of the amino acids in the tubules of the kidney is a competitive mechanism (39, 40, 41). More recently Orten and associates (42) working with a blind loop of human intestine have demonstrated that absorption of amino acids from the gastrointestinal tract is also competitive. There is no reason to believe that passage across a variety of cellular membranes does not take place in a like manner.

Nasset (43) has recently reported that the mixture of amino acids present in the gastrointestinal tract of dogs 90 minutes after feeding has an approximately constant composition regardless of the nature of the protein ingested. This observation tempts one to hypothesize that this is the body's mechanism for assuring presentation to the tissue of an amino acid mixture of the proper composition. Such a circumstance would necessitate the existence of a mechanism for elimination of the unused portions of the protein broken down to supply the amino acids in which the ingested protein was deficient. Further, it would necessitate that the pattern of amino acids in the plasma of the portal vein would also be constant regardless of the composition of the protein ingested. Since Denton and Elvehjem (44) have found this not to be the case but that the pattern of amino acids in the portal vein resembles that of the ingested protein, such a hypothesis is scarcely tenable.

The fact remains, however, that animals ingesting a high protein diet with consequent high protein reserves are not susceptible to the ill effects of amino acid imbalances. Whether these facts are cause and effect is unknown but not unlikely. It is also possible that high protein reserves might protect against other forms of stress such as infection. In this area the experimental data are not especially convincing. While numerous reports can be found to the effect that populations existing on low protein diets have a higher incidence of infectious diseases than those maintained on high protein intakes, many other uncontrolled factors such as sanitation and heredity may cast doubt on these conclusions. Unquestionably, animals subjected to severe prolonged protein undernutrition exhibit a pronounced loss of ability to form antibodies and resist infection (45, 46), but Stare and collaborators (47) were unable to demonstrate reduced resistance to natural infections in rats on a diet containing only 2% casein as the source of protein. More recently Dubos and Scheedler (48) reported that growing mice maintained on a diet containing 8% casein were much less resistant to a variety of bacterial infections than those receiving a 20% casein ration. Further, a diet containing 22% protein largely of vegetable origin was found to be scarcely more protective than the 8% casein diet and the former was greatly improved by the addition of amino acids of undisclosed identity. Comparatively little has been done in this field, and it may be that protein reserves even in adults will eventually be found to be highly beneficial in resistance to certain infections, as, indeed, it seems logical that they should be.

Evaluation of Proteins

If amino acid supplementation is to become an established practice, methods must be made available to determine the biological value of the protein to be supplemented and which amino acids and how much of each shall be added. The biological value may be established by any of a variety of methods which have been ably reviewed by Allison (49). However, to determine qualitatively and quantitatively by trial and error with biological methods which amino acid shall be used, is a formidable undertaking. Fortunately a first approximation is possible from the amino acid composition of the protein to be supplemented. Reliable assays are now available either by microbiological or chromatographic procedures.

Assuming that the amino acid composition of a foodstuff is known, a standard or ideal mixture must be available for comparison in order to estimate the limiting amino acids. Classically, whole egg protein has been used largely because under a variety of biological tests it is found to be almost 100% utilized. Nasset (50) has recently advanced evidence which appears to support the use of whole egg protein as a standard. By careful experiments he determined the mean quantity of each essential amino acid required for the maintenance of nitrogen balance in the rat when each of the others were present in excess. A mixture consisting of the essential amino acids in these proportions was then compared to one patterned after egg protein. Both mixtures were made isonitrogenous by the addition of L-glutamic acid. The mixture patterned after egg proved much the more effective. It however contained only 27% glutamic acid whereas 89% of any amino acid can be well balanced biologically. Further Nasset states that he used the mean requirements for making up the new mixture. This means that because of individual variations some animals did not receive adequate quantities of some of the amino acids and therefore showed poor nitrogen retention. These low results could not be offset by the performance of those animals which, because of individual variation, received more of these amino acids than was required since an excess above the optimum could not affect an already high nitrogen retention. The performance of the individual animals is not recorded and it is therefore impossible to know just how serious this matter may be.

It is submitted that whole egg protein is well utilized not because it possesses an ideal pattern of essential amino acids although its pattern is good, but in part because it contains such a high total percentage of them. An animal consuming a reasonable quantity of this protein will certainly receive adequate quantities of each of the essential amino acids and the excess of each will be used as nonspecific nitrogen. This does not mean that a mixture of amino acids patterned after egg protein is the most economical one. Almost certainly a portion of the essential amino acids present will be wasted as far as specific use as such is concerned. For this reason it is maintained that a mixture of amino acids based upon human requirements is the most logical standard of comparison. Attempts are now being made by the F. A. O. Committee on Protein Requirements to formulate and test such a mixture.

Fortification of Human Foods

In consideration of protein intake and consequently amino acid fortification of food it is well to remember that all populations may be divided into two categories: 1) Those who are in need of amino acids for synthesis of tissue protein as well as for tissue maintenance and 2) Those whose intake of amino acids is required for tissue maintenance alone. The first category includes children, pregnant and lactating women, and convalescents, the second, all other adults of both sexes. The amino acid requirements of these two groups differ both quantitatively and qualitatively (51, 52).

For a differing treatment of this subject and for an extensive bibliography the reader is referred to the recent work of Flodin (53, 54).

In Technically Developed Countries

In the highly industrialized areas of the Western World in which foods high in protein of good quality are readily available it would seem that amino acid deficiencies should not exist. However, a careful survey of the nutritional status of certain segments of these populations reveals that their nutrition may not be optimum.

Recently Albanese (55, 56) has reported a high percentage of infants who fail to respond to conventional formulas grow normally after the addition of lysine to their diets. Holt (57), on the other hand, reports investigations revealing the lysine requirement of the normal infant to be about 90 mg. per kilogram of body weight, much less than is supplied in the average diets. While much additional data are required to confirm Albanese's observations, it does not appear that the two findings are necessarily contradictory. It is possible that partial failure in digestion or absorption has limited the actual intake of lysine by the retarded infants and that additional free lysine corrects this deficiency. This possibility becomes more credible in view of the interesting results obtained by Orten (42). She has observed that an equimolecular quantity of tryptophan greatly reduces the absorption of lysine. A child suffering from diarrhea might not retain its food long enough to allow sufficient absorption of lysine especially if one or more other amino acids are retarding its absorption.

In a discussion of the nutritional adequacy of the diets of children of the United States Jeans (58) contends that based on creatinine excretion most children have a muscle mass below the theoretical normal. Creatinine output is a much better measure of protein mass than body weight. It is stated that when children are fed ample protein, the creatinine excretion and concurrently the amount of muscle rises to a constant level for each child, with a narrow range at each age period for a group of children. When these values are plotted according to the age and in terms of creatinine for each kilogram of body weight, a curve is obtained which may be considered as representing normal conditions as regards creatinine output and muscle mass. Jeans has found that a great majority of children studied have creatinine values below normal when they first come

under observation. In all instances, the creatinine output increases promptly to the normal level when adequate amounts of protein were fed. Thus it appears that the muscle mass of most children can be increased by feeding more protein.

Lynch and Snively (59) report that protein deficiency is prevalent among children largely because parents do not realize that proteins, like vitamins and calcium, are important. They contend that it is commonly believed that a child ingesting one quart of milk per day needs no other source of protein. This, of course, is not true. They list the symptoms of protein deficiency as follows: Anorexia, failure to gain weight, gastrointestinal disturbance, and a high incidence of infectious disease, dental caries and anemia.

If then, as appears possible many children in well developed countries may not be receiving adequate protein for optimum performance, one solution to the problem is to increase the intake of animal protein. For reasons of economy and poor eating habits this may not always be possible. If not, will amino acid supplementation increase the efficacy of the protein already being ingested? School children consume large quantities of snacks consisting largely of bread with low protein spreads. The value of lysine fortification of wheat protein is so firmly established that addition of lysine to these wheat protein based snacks would unquestionably yield a more nutritious food. It seems probable that some children would benefit from such a practice. On the other hand, Widdowson and McCance (60) report that the addition of milk, the proteins of which are high in lysine content, did not stimulate growth of children deriving a high percentage of their protein intake from wheat. One possible explanation of this seeming anomaly which appears to have been overlooked is that although the weight and height gains were identical the protein synthesis may not have been so. Allison (61) has shown that puppies will gain equally well on wheat gluten and whole egg but the gluten fed animals were "fat and soft while those fed whole egg were lean and hard." Increase of protein stores was much greater in the egg fed animals. Although Widdowson and McCance report that muscular tone was identical in both groups of their subjects, no nitrogen retention determinations were made. In a later communication these same investigators (62) report that the carcasses of young rats receiving a low protein diet have the same nitrogen content as those of similar animals ingesting much larger quantities of protein. The livers of the latter animals, however, were larger and contained a higher percentage of nitrogen. What is sorely needed are carefully controlled long term experiments in which the effect of lysine supplementation is determined not only upon nitrogen retention but upon the development, general health and well being.

In pregnancy the protein requirement for tissue synthesis is greatly increased. Burke and her associates (63, 64) state that a daily protein intake of less than 75 gm. during the latter part of pregnancy results in an infant who tends to be short, light in weight and likely to receive a low pediatric rating in other respects. They recommend a protein intake of 85 gm. per day but report that of those who come to the Boston Lying-in-Hospital only 10% receive this quantity while 38% receive less than 55 gm. daily. Since wheat proteins make up an appreciable part

of the protein intake of pregnant women it might be expected that lysine supplementation would be beneficial in a high percentage of cases. Such, indeed, appears to be the case in one area at least. Terry (65) gave 600 mg. lysine daily to 12 pregnant women who had consistently demonstrated hemoglobin levels less than 10.5 gm. per 100 cc. of blood. In each instance there was a prompt and often dramatic rise in hemoglobin level. This prompted him to study all obstetric cases seen by him at Dallas for one year. Lysine was given to 204 patients throughout pregnancy while 92 received only the usual prenatal treatment. The hemoglobin levels of those receiving lysine were uniformly higher than those of the control group, averaging at the end of the seventh month of pregnancy 11.8 and 10.2 gm. per 100 cc. respectively. Seven patients developed eclampsia none of whom were receiving the lysine supplement. In addition, the patients receiving lysine showed great subjective improvement, failing to a large extent to exhibit the lassitude and chronic fatigue so often encountered during the first trimester of pregnancy. More studies of this character are needed. It is well to show by dietary surveys that a population segment is on the average receiving more or less than the recommended intake of an essential nutrient and therefore suggest that supplementation is or is not desirable but the proof of the conclusions reached depends upon experimental demonstration.

Among the aged and aging there undoubtedly are some who for reasons of poor dentiture, poor eating habits or from necessity subsist to a great extent upon cereal diets. Within this group are many who suffer from osteoporosis. In osteoporosis there is a failure to maintain a proper protein matrix in the bone. Further, it has been reported recently (66) that lysine aids in the absorption of calcium. Possibly lysine supplementation would aid a portion of these subjects.

Ohlson and co-workers (67) state that the elderly are prone to suffer from protein deficiency more than from any other deficiency. Stieglitz (68) reports that the concentration of plasma proteins in the aged frequently reveals a moderate degree of protein insufficiency. Kountz and his associates (69) record cases of elderly persons who require extremely large quantities of protein (2 gm./kg./day) to maintain nitrogen balance and have become so depleted that when given such quantities they have continued in positive balance for a full year. Very recently Tuttle et al (70) have presented data showing that five men aged 52 to 68 could not be maintained in nitrogen balance on quantities of amino acid mixtures or egg protein which had previously been found to be adequate in young men.

Albanese *et al* (71) have reported the only study designed to determine whether amino acid supplementation would benefit the aged. They studied 20 women residing in a home for the aged on self selected diets, averaging 59 gm. of protein daily of which 39% was derived from meat. An increase in nitrogen retention was effected in 8 of the 20 by supplementation of their diet with from 200-250 mg. of lysine per day. In St. Luke's Hospital 39 elderly convalescents were found to consume, on a self selection basis, 64-98 gms. of protein daily of which meat protein constituted an average of 29%. One-half of these patients

were in negative nitrogen balance. Addition of 600-900 mg. of lysine daily was found to be accompanied by a significant improvement in nitrogen retention in 60% of the patients. The authors are aware that this increase may be due to general improvement in health, a possibility which is strengthened by the great increase in nitrogen retention observed in the post control period when no supplementary lysine was ingested.

These experiments are an indication that lysine supplementation in the aged may at times be beneficial. Again much more work must be done involving both biochemical and clinical evaluation of the subjects.

The individual essential amino acid requirements for nitrogen balance in young adults of both sexes have been determined (72, 73). These requirements are surprisingly low, totaling about six grams per day when a daily total of approximately ten grams of nitrogen is ingested. The minimum amount of non-specific nitrogen required has not been accurately determined, but probably lies between 2.28 and 2.55 gm. daily (74). The small amount of essential amino acids required does not mean that any diet which supplies these minimum quantities and the necessary amount of non-essential nitrogen will be an adequate one. We have already seen how easy it is to obtain an imbalance with low protein diets. It is, however, unlikely indeed in the technically developed nations of the Western World with their high animal protein intake, that any appreciable fraction of the adult population which fall in the second category mentioned earlier in this section, those which require nitrogen for maintenance only, are suffering from amino acid deficiencies.

In Underdeveloped Countries

As stated previously, in the technically underdeveloped countries probably two-thirds of the world's population subsists almost entirely on vegetable diets. In those limited areas in which legumes form a staple of the diet the amino acid intake resembles more closely that of the Western World since the legumes contain considerable quantities of the basic amino acids and adequately supplement the cereal proteins.

In those areas in which rice, corn, wheat, cassava, millet, etc., are the main dietary constituents there can be little doubt that amino acid supplementation would improve the nutritional status of children, pregnant women and convalescents. There is a plethora of evidence that lysine supplementation of wheat protein results in a product of increased biological value (53). The supplementation of the other cereals is not as clearly understood. As previously discussed added lysine and threonine will improve rice. Sauberlich, Chang and Salmon (75) have shown that high protein corn is improved by the addition of lysine and tryptophan but that to improve low protein corn such as is commonly grown in many areas of the world, lysine, tryptophan, isoleucine, threonine and valine are required. As mentioned previously it is not understood why the addition of lysine to rice and corn does not result in greatly improved products since analyses indicate lysine to be by far the most limiting amino acid in each case. However,

only recently Rosenberg and Culik (76) have shown that the addition of 0.5% lysine to diets of rats maintained largely on rice significantly increases their growth rate. Larger quantities have no effect or are deleterious. It would be most interesting to study the effect of the addition of low levels of lysine to the diets of children subsisting largely on rice. Although the amino acid compositions of most of the world's other staples have been determined, relatively little is known of their improvement by supplementation.

Widespread throughout those areas in which the population subsists largely on corn, cassava, banana and certain other low protein staples there exists a disease known as kwashiorkor. It is characterized by retarded growth and maturation, depigmentation of the hair, dermatitis, edema, apathy, anorexia and a variety of biochemical and physiological changes. It is apparently caused by an acute deficiency of protein and will respond to administration of skim milk (77) vegetable proteins (78) or amino acid mixtures (77, 79) with or without vitamins. It might be expected that this syndrome would yield to treatment with the amino acids which are low in the diet. This possibility is under test at the present time but it seems likely that a partial failure of digestion in the acute stages of the disease is a contributing factor (80). While amino acid fortification may fail to cure the disease, there is every possibility that used prophylactically before depletion of the protein reserves and subsequent failure of digestion, amino acids would prove singularly effective.

In that portion of the adult population which needs nitrogen only for maintenance the situation appears to be somewhat different. Hundley and his associates (81) studied the effect of amino acid supplementation on young adult males consuming a rice diet affording a daily protein intake of 26 to 32 gm. They found under these conditions the primary deficiency in rice is that of non-specific available nitrogen, that is, a mixture of non-essential amino acids was equally as effective as a mixture of those which are essential. Hegsted and co-workers (82) found that supplementation of the vegetable diets of young adult females receiving 25 gm. of protein per day with lysine and methionine improved the nitrogen retention following a depletion period. The authors conclude however "that considering the relative ease with which subjects approach nitrogen equilibrium on the unsupplemented diet, the likelihood of protein or amino acid deficiencies in an adult population consuming diets essentially of vegetable origin does not appear to be very great." In view of the results obtained by Hegsted and Hundley one is inclined to agree with this conclusion. However, its validity does not exclude the possibility that the protein stores of some adults could be increased by amino acid supplementation. In fact Hegsted's results indicate that this probably is the case. As previously discussed whether such a course of action would be beneficial must be determined by experimentation.

While it may be granted that large segments of the populations of the non-industrialized areas of the world would benefit from judicious amino acid fortification of their diets, it is unfortunately these very areas in which supplementation is most difficult. The economic barrier is a formidable one but even were it pos-

sible to surmount it such problems as distribution, overcoming local customs and changing cooking practices appear almost unsolvable. Certainly the problems encountered in the rice enrichment program in the Philippines indicate that widespread adoption of any such program in underdeveloped areas is likely to take time.

Fortification of Animal Feeds

As previously stated the rations of many farm animals consist largely of cereal grains, corn being the most widely used. It has been shown that cereal diets can be improved by addition of the proper amino acids. At present, however, these are now economically supplied by soy bean meal and animal and distillery by-products. Should the price structure change to favor the use of amino acids, a great deal of research remains to be done to establish that a diet consisting of corn, amino acids and other additives will be more practical than the mixed feeds now used.

At the present time, it is a common practice to supplement poultry feeds with methionine. This is accomplished by the addition of either DL-methionine, both isomers of which are utilized, or the alpha hydroxy-analog of methionine which, in the presence of a source of non-specific nitrogen, is equally as active as methionine itself. Approximately two million pounds of these materials are used in this country annually. If as suggested above, corn plus other amino acids should eventually replace the corn-soy mixtures now used, the requirement for methionine would probably decrease since corn has a proportionately higher content of this amino acid than does soy bean oil meal.

Recently it has been reported (83) that the protein level of a turkey starter feed may be reduced from 28 to 20 per cent by replacing animal protein with 0.2% lysine. Should the prices of lysine and animal by-products change appreciably this finding might create a considerable demand for lysine as a turkey feed additive.

The establishment of the value of amino acids as feed supplements is extremely complex. It must be remembered that while the nutritionists are determining the nutritional requirements of our existing livestock the animal breeders may be producing animals with different requirements and the plant breeders may be changing the nutritional value of the feed crops.

Summary

The potential for amino acid supplementation of human and animal feed is great. However, many factors combine to make it unlikely that this potential will be realized for many years. Since amino acid imbalances exist in nature it is logical that they be corrected but only after thorough study and evaluation.

In the United States and other technically developed countries, despite the high animal protein intake, it is likely that a certain percentage of children, pregnant and lactating women, convalescents and possibly elderly persons would

benefit from lysine supplementation of their diets. However, a great many more controlled experiments are needed to establish the extent of the benefit to be derived therefrom. It is unlikely that amino acid supplementation would benefit an appreciable percentage of other adults in the industrialized countries.

In underdeveloped countries in which whole populations exist almost entirely on protein of vegetable origin, amino acid supplementation would undoubtedly benefit high percentages of these people. Nonetheless, much more research is necessary before such a program could be undertaken. Even were all technical facts known there are many almost insurmountable barriers to widspread supplementation. These include cost, distribution and overcoming customs and cooking prejudices.

Methionine is presently being used as a poultry feed supplement. Much more information and a change in price structure of feeds and amino acids will be necessary before there is much greater use of amino acids in animal feeds.

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